Significant Effects of Mild Endogenous Hormonal Changes in Humans: Considerations for Low-Dose Testing

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We review the significant and adverse health effects that can occur with relatively small endogenous hormonal changes in pubertal and adult humans. We discuss the effects of hormonal changes that occur within normal physiologic ranges—such as the rising levels of estrogen in peripuberty, which cause growth spurts at low levels and then the fusion of epiphyses at higher levels—and the hormonal variations during the menstrual cycle and their relation to genital phenotypic changes and intercurrent disease evolution. We turn next to adaptive changes in gonadal and other functions during aging, exercise, stress, starvation, and chronic diseases, which can serve as models for the effects of exogenous, hormonally active compounds. Then we review the states of borderline hormonal imbalances such as subclinical (having few or very mild symptoms, if any) hypothyroidism or hyperthyroidism, glucose intolerance, and other endocrine conditions. Finally, we review the deleterious systemic effects of gonadal imbalance. Information stemming from clinical observations leads to the concept of "no threshold" within the endocrine system and thus illustrates the importance of considering low-dose testing for chemicals that interfere with hormonal activity. We also urge attention to more sensitive, less visible end points such as osteoporosis, increased risk for cardiovascular disease, or cognitive changes. *Key words*: adverse effects, aging, endocrine disruptors, estrogen, exercise, fertility, glucose intolerance, hyperthyroidism, hypothyroidism, low dose, puberty, reproduction, starvation, stress, testing, threshold. — *Environ Health Perspect* 109(suppl 1):21–26 (2001).
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The endocrine system controls harmonious development, growth, and homeostasis. Although the range of normal values for hormones is often quite wide, certain ones, such as the steroidal hormones, function physiologically at extremely low concentrations, in the nanomolar (parts per billion) to picomolar [parts per trillion (ppt)] range. It is well established in clinical science that even mild hormonal imbalances can be associated with significant adverse health effects. Examples of health outcomes from mild endocrine perturbation can be instructive for understanding the complexity of testing hormonally active compounds. This is especially true when evaluating whether environmentally relevant low doses of synthetic chemicals can have biologic effects and when considering the appropriateness of applying toxicologic thresholds to hormonally mediated events.

One of the most controversial issues in toxicology is whether exposure to low doses of endocrine-disrupting chemicals (EDCs) can cause adverse health consequences. The term *endocrine-disrupting chemicals* here refers to synthetic or natural chemicals that mimic or interfere with the natural hormones that control development, reproduction, and function. Unfortunately, much of this debate remains theoretical because existing data are scant, controversial, and limited to a small number of chemicals (*1*). Furthermore, defining what constitutes a low dose is a challenge because such a definition depends on the context (toxicologic, pharmacologic, physiologic, or environmental). To some extent, clinical thresholds in endocrinology may provide guidance for evaluating toxicologic thresholds because they can show where perturbation in hormonal levels may not be inherently adverse but can be a risk factor for the development of clearly adverse health outcomes.

To address these problems, we examine the literature relating to endocrine systems in pubertal and adult humans. We first describe examples of shifts in normal hormonal values associated with a physiologic change in phenotype. We then show the adaptive endocrine consequences of age, stress, nutrition, exercise, or chronic disease. Next, we focus on the adverse consequences of mild endocrine diseases in pubertal and adult humans and on the deleterious systemic effects of gonadal imbalance. We then discuss the different thresholds in medicine—diagnostic or therapeutic—leading to the no-threshold concept pertaining to the specificity of the endocrine systems. Building on these facts, we address the implications for testing chemicals for their endocrine impact, with special attention to low-dose testing and specific end points.

Hormonal Changes within Physiologic Ranges Causing Phenotype Modification

Role of Estrogen Before and During Puberty

Estrogen has a biphasic effect on epiphyseal growth. Whereas low levels of estradiol (E_2) favor the pubertal growth spurt, higher pubertal levels cause fusion of the epiphyses, thus stopping growth (*2*). The administration of a low dose of E_2 increases serum concentration from < 1 pg/mL to 4 pg/mL (ppt) and increases prepubertal growth rates in both boys and girls by more than 60%, suggesting that the optimal growth-promoting effect of E_2 occurs at 4 pg/mL (*2*). By way of comparison, adult levels of E_2 range from 20 to > 200 pg/mL in women and are < 50 pg/mL in men. Thus, prepubertal bone plates are exquisitely sensitive to E_2, responding maximally at levels up to 50 times lower than adult levels.

The recent development of ultrasensitive assays for measuring hormone levels has demonstrated that prepubertal girls have levels of E_2 eight times higher than those of prepubertal boys (0.6 ± 0.6 pg/mL vs. 0.08 ± 0.2 pg/mL), and this gap widens in the peripubertal period (*2*). Sex differences in the timing and duration of the pubertal growth spurt are attributed mostly to the earlier increase and sharper rise in E_2 levels that occur in girls. Because girls reach the significant level of 4 pg/mL E_2 earlier than boys, they have an earlier growth spurt. Because the levels of E_2 rise sharply, fusion of epiphyses also occurs earlier in girls, creating a shorter growth spurt and lower final height compared to boys (*2*).

Hormonal Changes Occurring during the Menstrual Cycle

The menstrual cycle is a physiologic process that occurs regularly from puberty to menopause. As a result, the ovary secretes

variable amounts of estradiol and progesterone under tight hypothalamo-pituitary control (*3*). There are two distinct phases: The follicular phase is characterized by rising levels of estrogens and low levels of progesterone, leading to the maturation of a dominant follicle and ovulation, announced by a peak of luteinizing hormone (LH). The luteal phase prepares the uterus for potential implantation. The phenotypic consequences during these two phases depend on concentrations and timing (*3*). Indeed, the marked changes in hormonal levels, albeit within physiologic ranges, cause important modifications in target organs such as the vagina, uterus (already evident on a simple pap smear), ovary, and breast, as well as changes in thermoregulation (*4,5*).

It would be easy to overlook the effect(s) of EDCs because of the vulnerability of the endocrine system to stressors unrelated to EDC exposure as well as underlying conditions. Sexual difference is obvious for some diseases, with incidence greater in women than in men; furthermore, the incidence of disease seems influenced by the woman's hormonal status (puberty, pregnancy and postpartum period, or menopause). This is particularly true for autoimmune diseases such as Graves disease or Hashimoto thyroiditis (*6*), as well as rheumatoid arthritis and systemic lupus erythematosus, for example (*7*). It is well known that pregnancy is associated with change in immune response, followed by a frequent rebound postpartum, depending on the type of autoimmune disease (*6,7*). These changes in the immune system are likely due to a shift in cytokine balance secondary to hormonal changes (estrogen, progesterone, and/or cortisol) (*7*).

Furthermore, clinicians have long recognized that some medical conditions, such as migraines, epilepsy, asthma, and even diabetes control, are influenced by normal menstrual cycles (*8*). For example, it is possible that estrogen, through its actions on the hippocampus, could affect seizure activity (*9*). Although the possible protective effect of estrogens against Alzheimer's disease is under study, it has also been suggested that mild variations in cognitive function occur during the menstrual cycle (*10*). These effects are not global but target verbal memory and sexually dimorphic cognitive skills such as verbal articulation and fine motor skills. However, these effects have been identified in an experimental laboratory setting and their clinical significance is debatable (*10*). Finally, the premenstrual syndrome includes an array of cyclical mood disorders that have been linked to alterations in serotonergic activity in the central nervous system related to the menstrual cycle (*11*). In this case, the respective influence of different hormones is currently unknown.

Changes in Endocrine Function under Changing Conditions

Reproduction is particularly sensitive to endogenous and exogenous disruption in both sexes, but disruption has been documented more frequently among women. Additionally, it has been demonstrated (*12*) that the gonadal axis is highly sophisticated, with a precise set of regulatory mechanisms, whereby its function changes under certain conditions. Current knowledge about several adaptations to changing conditions can provide models of what might occur as a result of exposure to EDCs. For example, if a woman is very young or very old (*13–21*), exercises intensely (*22–23*), has not enough food (*24–29*), is stressed (*30–33*), or is sick (*28,34–38*), the delicate balance of the hypothalamo–pituitary–ovarian axis is altered, impairing or suppressing ovulation. Thus, from an adaptive point of view, it is clear that there is no advantage for a woman to be pregnant in situations where the outcome for the mother or the fetus is not optimal.

Age

The hormonal changes occurring during a woman's reproductive life span are well known. Menstrual irregularity is common at both ends of a woman's reproductive life. In adolescence, menstrual irregularities may stem from an immature gonadal axis (*13*). In perimenopause, the irregularity reflects progressive ovarian failure and is associated with a dramatic decrease in fertility (*14*). In adolescence and premenopause, the suboptimal hormonal setting may produce poorer pregnancy outcome, even though in teenage mothers the outcome also may be influenced by adverse economic circumstances (*15*). There is a U-shaped relationship between maternal age and low birth weight: Women under 15 and above 40 years of age are at higher risk than women between 25 and 29 years of age (*15*).

Fecundity of women of advanced reproductive age has been studied specifically because of the sociologic changes that have led many women to postpone starting a family. Recent work (*16–19*), boosted by the research in medically assisted techniques, has allowed a better understanding of the difficulty to conceive. The premenopausal changes are associated with a rise in follicle-stimulating hormone (FSH) levels and a decline in ovarian inhibin B secretion. Interestingly, fertility becomes compromised even before cycle irregularity, likely because of oocyte dysfunction (*16*). Some additional decrease in uterine function has been suggested (*17*) that can cause decreased implantation and pregnancy loss. Experience from

in vitro fertilization in women of advanced reproductive age shows that the rate of successful pregnancy decreases after 35 and more sharply after 40. Studies with oocytes from young women donors suggest that oocyte dysfunction is indeed the primary culprit (*18*). Endocrine markers have been used to predict fecundity in women of advanced reproductive age. For example, single measurements of estradiol (> 80 pg/mL) and FSH (> 13 mIU/mL), as measured at day 3 of the menstrual cycle, could have useful negative prognostic value (*19*).

Pregnancy in older women is more often complicated by glucose intolerance and hypertension (*20*). This, of course, has deleterious consequences on the fetus: Indeed, globally the perinatal outcome seems worse than in women younger than 40 (*21*). Preterm delivery was observed more frequently (18.5 vs. 11.7%), and low and very low birth weight occurred more often. The prevalence of macrosomia was also higher (8 vs. 4.8%), probably because of the increased incidence of glucose intolerance. Finally, newborn mortality (3.3 vs. 1.6%) and morbidity (20.4 vs. 11.4%) were increased (*21*).

Exercise

Chronic extensive exercise is well known to alter the menstrual cycle (*22*), though with marked individual variation. Menstrual disorders resulting from exercise include delayed menarche, short or inadequate luteal phases, and even secondary amenorrhea. This can cause infertility, which may be reversible, however, when training ceases. Exercise alters the pulsatile release of gonadotropin-releasing hormone (GnRH) and induces lasting increase in cortisol and endorphins. These changes may be viewed as a functionally adaptive phenomenon—an energy-saving strategy to protect more important biologic processes. Indeed, basal metabolic rate and caloric intake increase during the luteal phase, so there is a metabolic cost of maintaining an ovulation and an advantage of eliminating it under certain circumstances (*22*). In addition, some alterations in thyroid function are induced by exercise (*23*). Chronic intense training in women athletes with menstrual cyclicity is accompanied by an isolated decrease in thyroxine (T4) levels. However, in athletes with amenorrhea, all the thyroid hormones [T4, free T4, tri-iodothyronine (T3), free T3, and reverse T3 (rT3)] are decreased, whereas thyroid-stimulating hormone or thyrotropin (TSH) is normal in basal condition but is less responsive to a thyrotropin-releasing hormone (TRH) challenge. This suggests a central mechanism, including an impairment of the hypothalamo–pituitary–thyroid axis along with the hypothalamo–pituitary–ovarian axis during chronic exercise (*23*).

Starvation

Starvation or reduced caloric intake causing a negative energy balance disrupts the gonadal axis. In women, delayed menarche and primary or secondary amenorrhea are classic in anorexia nervosa and starvation, although anorexia nervosa is a complex disorder that involves both nutritional and psychologic components that can affect the gonadal axis (24).

The negative impact of low caloric intake on reproduction may be seen as a homeostatic adjustment that restricts unnecessary energy expenditure (25). The endocrine effects include an increase in corticotropin-releasing factor (CRF), with its negative effect on gonadotropins (particularly LH pulsatility), a decrease in TRH synthesis, and an increase in growth hormone (GH) secretion. The mechanism of increase in GH may not be central because insulin-like growth factor is normal or low, suggesting a more peripheral defect (24). Starvation and fasting also quickly induce a decrease in TRH and T3 levels (T3 and rT3) (26), with a change in deiodinase activity. These hormonal changes are corrected by the administration of leptin, a hormone secreted by adipose tissue, which is a barometer of adipose tissue activity (25).

There is a link between the gonadal axis and body fat content (24) whereby gonadal dysfunction occurs mainly under a specific weight threshold (loss of 10–15% of body weight) (27). Poor nutritional status is also linked to increased risk of maternal mortality and low birth weight, and malnourished populations are more susceptible to chronic illnesses that may also affect reproduction (28). Experimental work helps clarify the mechanisms involved. For example, in male rats, complete fasting for 4 days decreases LH and FSH by 30% and testosterone serum levels by 42%. Furthermore, the suppression of pituitary–testicular function is reversed by pulsatile GnRH substitution (29).

Stress

Stress is another factor with a strong impact on the gonadal axis because it causes an increase of CRF and β-endorphin in the hypothalamus, inhibiting gonadotropins, oxytocin, and vasopressin (30). In addition, glucocorticoids suppress the secretion of LH by the pituitary and of estradiol and progesterone by the ovary, and induce target tissues resistant to estradiol (31). In women, cycling disturbance or even amenorrhea can occur during stress, as can impairment of parturition or lactation (30). Prevention of conception could be viewed as an advantage at a time when the environment may be hostile to the survival of both mother and young (30). In men, evidence suggests that mild-to-severe emotional stress reduces testosterone levels and perhaps interferes with spermatogenesis (32). The decrease in testosterone may be a direct effect of glucocorticoids on testosterone biosynthesis (33).

Chronic Disease

Gonadal dysfunction is frequent in men and women with systemic disorders such as renal failure, liver cirrhosis, sickle cell anemia (34), chronic respiratory disease, gastrointestinal disease, or cancer (35), and with severe infections such as malaria or pneumonia (28). The mechanisms involved are often multiple (hypoxia, altered hormonal clearance, fever, nutritional status, or drugs). Acute illness (brain trauma, myocardial infarction, or elective surgery) is usually associated with temporary hypogonadotropic hypogonadism (35). Both primary and secondary hypogonadism may occur (35). In addition, the euthyroid sick syndrome (low T3, normal or low T4, and normal TSH) is common in chronic disease, and its degree often correlates with the seriousness of the underlying disease (36).

Deleterious Effects of Hormonal Imbalance in Adults

Normal ranges for hormone levels are usually defined as those levels not associated with pathologic symptoms. These ranges are used to set thresholds convenient for clinical use and diagnostic or therapeutic decisions. However, as is true for most or all physiologic functions, a continuum connects the physiologic and pathologic levels, which often overlap. This explains the development of dynamic tests that assess the response to hormonal challenge, though they are also bound by arbitrary criteria of normal responses. In this section we describe some of the health outcomes associated with relatively mild hormone perturbation. In addition, it is well established that menstrual cycles and sexual function may be indirectly disturbed in many endocrine conditions—for example, hypo- or hyperthyroidism (37), diabetes (38), and pituitary tumors that secondarily affect the gonadal axis. This can occur in women as well as in men (34,35) and illustrates the interrelationship of the different endocrine systems. However, the individual susceptibility to gonadal imbalance caused by other endocrinopathies is variable. For example, some women have adverse gonadal effects caused by only mild endocrine disease, whereas others are still fertile despite major imbalance.

Borderline Hypo- and Hyperthyroidism

Longstanding borderline or mild hypo- or hyperthyroidism has been linked to potential adverse health effects, and therapy for these disorders is generally recommended (39). Subclinical or mild hypothyroidism is common in women and in the elderly and has been associated with alteration in the lipoprotein profile, such as increased cholesterol, and with increased incidence of depression by lowering of the threshold for the development of major depressive disorders (40) and other mood disorders (41,42). In addition, mild hypothyroidism has been linked with diminished response to standard psychiatric treatment and with cognitive dysfunction (41). A 0.26 ng/dL geometric mean difference in free T4 in women during the second trimester of pregnancy has been associated with an average 4-point IQ reduction in their children (0.97 ng/dL vs. 0.72 ng/dL; $p < 0.001$) (43). Cases ($n = 62$) were selected from among 25,216 pregnant women with serum TSH levels at or above the 98th percentile with matched controls ($n = 124$). These results reveal the sensitivity of brain development to thyroid hormones.

Subclinical hyperthyroidism, which can be observed in patients with toxic thyroid nodule or on suppressive thyroid hormone therapy, may cause bone demineralization, particularly in postmenopausal women (40,44). Cortical bone is more affected than trabecular bone, but it is uncertain whether the overall risk of fracture is increased (45). Mild hyperthyroidism also confers a 3-fold relative risk for the development of atrial fibrillation (40). In addition, other cardiac changes have been reported in middle-aged patients (average age 39 years) on suppressive therapy (46): increased heart rate, premature atrial contractions, changes in left ventricular mass, and enhanced velocity of ventricular shortening. At this point, it is unclear whether these changes have deleterious clinical consequences (44).

Glucose Intolerance

Glucose intolerance is an intermediate category between normal glucose tolerance and overt diabetes and represents a gray area between normal and clearly pathologic glucose homeostasis associated with the risk of specific diabetic complications. A dynamic test such as a glucose challenge test is needed to diagnose this intermediate state. Glucose intolerance is common, with an incidence varying widely (2–25%) among populations (47). It is a risk factor for atherosclerosis (48) and overt diabetes. It is also associated with insulin resistance, which is part of the metabolic syndrome that includes hypertension, central obesity, hyperinsulinemia, and hyperlipidemia (48). Some preliminary data suggest an association between glucose intolerance and autonomic neuropathy (49).

Mild Hyperprolactinemia

Mild hyperprolactinemia has a negative significant impact on the gonadal axis in both women and men. Prolactin is mildly elevated in primary hypothyroidism, polycystic ovaries,

and stress, in patients using neuroleptic and other antidopaminergic drugs, in patients with acromegaly or pituitary macroadenomas compressing the infundibulum, and in patients with renal failure (*50*). Often no specific cause is found and this hyperprolactinemia is called *functional*. Mild hyperprolactinemia has also been linked to an increased risk of autoimmune disease (*51*).

Mild Hyperandrogenism

Mild increased secretion of androgens is the most common endocrinopathy in women, affecting 10–20% of women (*52*). It originates from the adrenal gland or the ovary. Beyond the cosmetic aspects of acne, hirsutism, androgenic alopecia (male-pattern hair loss) and their psychologic consequences, this hyperandrogenism may disrupt normal gonadal function and cause menstrual irregularity. The degree of menstrual irregularity is directly linked to the degree of hyperandrogenism. In fact, there is a direct correlation between testosterone levels and the length of the follicular phase, and an indirect correlation with the length of the luteal phase (*53*). Hyperandrogenic women with polycystic ovarian syndrome experience obesity, various degrees of insulin resistance, and unfavorable lipid patterns (*52*). Interestingly, in a significant subgroup of women with clinical hyperandrogenism, androgen levels are normal, suggesting an increased end-organ sensitivity to androgens (*52*). This is important because for this subgroup of subjects, the observed effect may come from receptor dysfunction or postreceptor events, not from hormonal imbalance *per se*.

Deleterious Health Consequences of Gonadal Imbalance

Beyond the evident effects on reproduction, there also are systemic negative effects of gonadal imbalance. One of the most studied effects today is the increased risk of osteoporosis (*54*). The occurrence of hypoestrogenic amenorrhea in young women prevents the reaching of peak bone mass and later leads to an increased risk of osteoporosis (*55*) or even fractures (*54*). Specifically, adolescent girls with oligomenorrhea and amenorrhea, whether linked (*56*) or not (*57*) to exercise or eating disorders, are at risk for osteopenia. Interestingly, cyclic treatment with a progestative (medroxyprogesterone) to mimic normal cycles in women with irregular cycles may increase bone density (*58*). These preliminary data suggest that not only estrogen *per se* but also an optimal ratio of estrogen to progesterone could significantly help prevent osteoporosis in premenopausal women. Hypoestrogenic amenorrhea is also associated with adverse alterations of the lipoprotein

profiles and consequently an increased risk of cardiovascular events (*54*).

The beneficial effects of estrogens on cognition and mood have also recently been recognized in postmenopausal women (*59*). More strikingly, breast, ovarian, and endometrial uterine cancers are all recognized as hormone-dependent cancers (*60*). Mild hormonal imbalance, exogenous hormonal administration (oral contraception or estrogen replacement therapy after menopause), and sheer physiologic events such as the occurrence of pregnancies or the age at first menses are involved, showing that in those cases there may be no threshold for the risk of cancer. In breast cancer, for example, epidemiologic studies (*60–62*) have shown that the risk is linked to different parameters integrating the lifetime duration of exposure to estrogens (i.e., age at first menses, menopause, and pregnancies). Inadequate luteal levels unable to oppose estrogen levels may also be a factor (*61*). Interestingly, pregnancy seems to have two discordant effects on the risk of breast cancer. Epidemiologic studies have shown that a completed pregnancy seems to decrease the risk, whereas first-trimester interrupted pregnancies are associated with an increased risk (*60*). A more recent study demonstrates that the dual effect of pregnancy could be explained by the long-term protective effect against cancer and a short-term increase in risk after each pregnancy (*62*). In addition, it is suggested that the higher risk of postmenopausal breast cancer in obese women is linked to the aromatization of androgens into estrone in the adipose tissue (*61*). Differences in international rates of breast cancer have also been linked to differences in estrogen levels (*61*). Ovarian cancer is more frequent in infertile or nulliparous women, whereas the use of contraceptive pills is associated with a lesser risk (*63*). These results led to the theory of "incessant ovulation" for ovarian carcinogenesis (*64*). Finally, it is well established that endometrial carcinoma is a hormonally determined disease, usually occurring after menopause (*63*). In addition, anovulatory amenorrhea, with its hormonal profile characterized by very low levels of progesterone and subnormal levels of estrogen, increases the risk of endometrial hyperplasia and carcinoma even in young women (*54*). Of course, other risk factors are involved, such as genetic predisposition or diet (*60*).

Debate over estrogen replacement therapy after menopause with doses supposed only to expand the duration of normal female estrogen and progesterone exposure is often heated because treatment seems to carry both benefits and risks. Treatment decisions usually stem from the intensity of menopausal symptoms, risk factors for hormonal cancer and osteoporosis, a woman's preference, and also

the physician's attitude. The difficulty of giving a clear answer to a given woman illustrates well the controversy linked to hormonal effects, even when replacement targets are within physiologic ranges.

Medical Thresholds

The concept of threshold or of a variable threshold is used routinely in medicine when criteria are set for diagnosis and therapeutic intervention. Determination of a threshold is complicated because typically there is overlap in clinical values such that affected persons may have values within the normal range and unaffected persons may have values outside the normal range. Therefore, diagnostic threshold criteria are relatively arbitrary cutoff values, representing a balance between the ability of the screen to detect truly affected persons (sensitivity) and the appropriate categorization of nonaffected persons (specificity).

Continuous variables such as blood glucose (*65*) or cholesterol (*66*) illustrate well the continuum in the risk associated with a given parameter. We have already discussed the relationship between glucose tolerance and cardiovascular disease. Indeed, several epidemiologic studies show that there is no threshold for the link between glycemia and cardiovascular disease (*65,67*). The authors of the Framingham Offspring Study concluded, "Metabolic risk factors for Type 2 diabetes mellitus and for cardiovascular disease worsen continuously across the spectrum of glucose tolerance categories, beginning in the lowest quintiles of normal fasting glucose level" (*65*).

Diagnostic procedures and the decision to proceed with therapeutic intervention, which defines therapeutic thresholds, are also influenced by other factors, including life stage and the presence of other risk factors. For example, during pregnancy, the screening for gestational diabetes uses both different test and cutoff values of blood glucose (Table 1) (*68*) because during pregnancy the outcome of the fetus also must be taken into account. Gestational diabetes is associated with significant maternal and fetal morbidity and long-term consequences for the offspring (increased weight, glucose intolerance, or even poorer intellectual performance, and psychomotor development) (*69–71*). Fetal and neonatal hyperinsulinemia in offspring of diabetic mothers causes immediate neonatal hypoglycemia and subsequent development of childhood obesity and glucose intolerance (*69*). In addition, although criteria for determining hypertension are highly debated (*72*), the criteria for treating hypertension are also more stringent in diabetic than in nondiabetic patients (*73*). Thresholds for therapeutic interventions should indeed be tailored for some groups of patients. In summary, there may be no threshold for a given risk; but

Table 1. Screening protocols for diabetes outside and during pregnancy.

Protocols	Outside pregnancy	During pregnancy	
		Screening test	Confirmatory test
Conditions of OGTT			
Dose of glucose	75 g glucose	50 g glucose	100 g glucose
Conditions of test	Fasting	Random	Fasting
Duration of test	2 hr	1 hr	3 hr
Criteria for diagnosis of diabetes	Glycemia[a] at 2 hr ≥ 2 g/L	Glycemia[a] at 1 hr ≥ 2 g/L If glycemia ≥ 1.3 g/L, possible diabetes; → confirmatory test	At least 2 glycemia[a] measurements at or above fasting 0.95 g/L; 1 hr 1.80 g/L; 2 hr 1.55 g/L; 3 hr 1.40 g/L

OGTT, oral glucose tolerance test.
[a]All glycemia readings are plasma glucose measurements.

from a practical point of view, thresholds are set both for diagnostic and therapeutic purposes. The history of medicine has shown that their fate is to be revisited in the light of new science.

What is well recognized for parameters such as blood glucose, lipids, or blood pressure mentioned above is also quite valid for hormonal levels in general. Indeed, in some cases, adverse effects occur when hormone levels are still in the normal ranges defined for a population but are in fact elevated for a given individual. This is illustrated by the occurrence of suppressed TSH (a sign of hyperthyroidism) associated with levels of thyroid hormones in the upper side of the normal range. Furthermore, when one assesses a risk for a given individual or population, one should also consider other risk factors and use clinical judgment. For example, in geographic regions of iodine deficiency, individuals with a genetic predisposition to thyroid disease may not be able to compensate for an additional thyroid stressor. In addition, chemicals with the potential to disrupt thyroid homeostasis may have more significant health effects in such regions. In this respect, clinical experience may provide guidance for decision making regarding EDCs.

Implications for Testing of Endocrine-Disrupting Chemicals

The application of the threshold assumption, commonly used in toxicology, to endocrine-disruptor testing may be problematic because the endocrine system at baseline has already achieved physiologic threshold (the effects observed with hormones within the physiologic range), which is the basis of normal hormonal function. Therefore, if a risk is already associated with background endogenous levels of hormones, then a threshold for an adverse effect may already have been reached, and any additional hormonally active compound is potentially harmful, at least for a susceptible subgroup of the population (*74*). This may well be the case for the risk of breast cancer. The notion of individual susceptibility is important and encompasses many factors, e.g., genetic susceptibility to cancer or autoimmune disease, receptor function, environmental factors such as diet (fat, iodine content) or chemical exposure. Recently, the no-threshold hypothesis was tested with turtle eggs, in which sex determination normally is temperature dependent but can be altered by exogenous estrogen exposure. In this study, even the lowest dose of estradiol applied to eggs (400 pg/egg or ~ 40 ng/kg) resulted in sex reversal in 14.5% of the eggs (*74*).

In light of the clinical experience in endocrine disease reviewed above, we consider that traditional toxicologic low-dose testing (at best in the parts per million range), although appropriate for testing carcinogenic effects, may not be suitable for determining endocrine effects. Furthermore, although it is difficult to assess and extrapolate from one species to another, it is also important to expand the study spectrum to more sensitive end points, especially those relevant to endocrine effects. This could include, for example, bone mineralization, relevant for thyroid, gonadal, and adrenal systems; detailed cardiac and metabolic function; and behavior and cognitive functions.

In our view, the data presented for adults are already compelling. It is likely that the human embryo and/or fetus, having less built-in compensatory capacity than an adult, may often be more vulnerable to any exogenous or endogenous endocrine disturbance. Indeed, the placenta does not provide the protection that was assumed in the past. This is illustrated by measurements of detectable levels of synthetic chemicals in cord blood, reflecting placental transfer. Consequently, special attention is required when testing endocrine developmental toxicity.

REFERENCES AND NOTES

1. U.S. EPA. Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) Final Report: Vols I and II. Washington, DC:U.S. Environmental Protection Agency, 1998.
2. Cutler GB Jr. The role of estrogen in bone growth and maturation during childhood and adolescence. J Steroid Biochem Mol Biol 61:141–144 (1997).
3. Franz WB III. Basic review: endocrinology of the menstrual cycle. Prim Care 15:607–616 (1988).
4. Bates GW, Garza DE, Garza MM. Clinical manifestations of hormonal changes in the menstrual cycle. Obstet Gynecol Clin North Am 17:299–310 (1990).
5. von Schoultz B, Soderqvist G, Cline M, von Schoultz E, Skoog L. Hormonal regulation of the normal breast. Maturitas 23(suppl):S23–S25 (1996).
6. Case AM, Reid RL. Effects of menstrual cycle on medical disorders. Arch Intern Med 158:1405–1412 (1998).
7. Woolley CS, Schwartzkroin PA. Hormonal effects on the brain. Epilepsia 39(suppl 8):S2–S8 (1998).
8. Sherwin BB. Estrogenic effects on memory in women. Ann NY Acad Sci 743:213–230; discussion 230–231 (1994).
9. Bancroft J. The menstrual cycle and the well being of women. Soc Sci Med 41:785–791 (1995).
10. Chiovato L, Lapi P, Fiore E, Tonacchera M, Pinchera A. Thyroid immunity and female gender. J Endocrinol Invest 16(5):384–391 (1993).
11. Wilder RL. Hormones, pregnancy, and autoimmune diseases. Ann NY Acad Sci 840:45–50 (1998).
12. Rabinovici J. The differential effects of FSH and LH on the human ovary. Bailliere's Clin Obstet Gynaecol 7(2):263–281 (1993).
13. Mansfield MJ, Emans SJ. Adolescent menstrual irregularity. J Reprod Med 29:399–410 (1984).
14. Li S, Lanuza D, Gulanick M, Penckofer S, Holm K. Perimenopause: the transition into menopause. Health Care Women Int 17(4):293–306 (1996).
15. Reichman NE, Pagnini DL. Maternal age and birth outcomes: data from New Jersey. Fam Plann Perspect 29(6):268–272, 295 (1997).
16. Klein NA, Soules MR. Endocrine changes of the perimenopause. Clin Obstet Gynecol 4:912–920 (1998).
17. Fitzgerald C, Zimon AE, Jones EE. Aging and reproductive potential in women. Yale J Biol Med 71:367–381 (1998).
18. Sauer MV. Pregnancy wastage and reproductive aging: the oocyte donation model. Curr Opin Obstet Gynecol 8:226–229 (1996).
19. Buyalos RP, Daneshmand S, Brzechffa PR. Basal estradiol and follicle-stimulating hormone predict fecundity in women of advanced reproductive age undergoing ovulation induction therapy. Fertil Steril 68(2):272–277 (1997).
20. Lagrew DC Jr, Morgan MA, Nakamoto K, Lagrew N. Advanced maternal age: perinatal outcome when controlling for physician selection. J Perinatol 16:256–260 (1996).
21. Pugliese A, Vicedomini D, Arsieri R. Perinatal outcomes of newborn infants of mothers over 40 years old. A case-control study. Minerva Ginecol 49(3):81–84 (1997).
22. Bonen A. Exercise-induced menstrual cycle changes. A functional, temporary adaptation to metabolic stress. Sports Med 17:373–392 (1994).
23. Loucks AB, Laughlin GA, Mortola JF, Girton L, Nelson JC, Yen SS. Hypothalamic-pituitary-thyroidal function in eumenorrheic and amenorrheic athletes. J Clin Endocrinol Metabol 75:514–518 (1992).
24. van der Spuy ZM. Nutrition and reproduction. Clin Obstet Gynaecol 12(3):579–604 (1985).
25. Judd SJ. Disturbance of the reproductive axis induced by negative energy balance. Reprod Fertil Dev 10:65–72 (1998).
26. Danforth E Jr, Burger AG, Wimpfheimer C. Nutritionally-induced alterations in thyroid hormone metabolism and thermogenesis. Experientia Suppl 32:213–217 (1978).
27. Frisch RE. The right weight: body fat, menarche and ovulation. Bailliere's Clin Obstet Gynaecol 4:419–439 (1990).
28. Mascie-Taylor CG. Endemic disease, nutrition and fertility in developing countries. J Biosoc Sci 24:355–365 (1992).
29. Bergendahl M, Perheentupa A, Huhtaniemi I. Starvation-induced suppression of pituitary-testicular function in rats is reversed by pulsatile gonadotropin-releasing hormone substitution. Biol Reprod 44(3):413–419 (1991).
30. Laatikainen TJ. Corticotropin-releasing hormone and opioid peptides in reproduction and stress. Ann Med 23(5):489–496 (1991).
31. Chrousos GP, Torpy DJ, Gold PW. Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann Intern Med 129(3):229–240 (1998).
32. McGrady AV. Effects of psychological stress on male reproduction: a review. Arch Androl 13(1):1–7 (1984).
33. Schaison G, Durand F, Mowszowicz I. Effects of glucocorticoids on plasma testosterone in men. Acta Endocrinol (Kbh) 89:126–131 (1978).
34. Morley JE, Melmed S. Gonadal dysfunction in systemic disorders. Metabolism 28(10):1051–1073 (1979).
35. Turner HE, Wass JA. Gonadal function in men with chronic illness. Clin Endocrinol 47(4):379–403 (1997).

36. McIver B, Gorman CA. Euthyroid sick syndrome. Thyroid 7(1):125–132 (1997).

37. Koutras DA. Disturbances of menstruation in thyroid disease. Ann NY Acad Sci 816:280–284 (1997).

38. Adcock CJ, Perry LA, Lindsell DR, Taylor AM, Holly JM, Jones J, Dunger DB. Menstrual irregularities are more common in adolescents with type 1 diabetes: association with poor glycaemic control and weight gain. Diabet Med 11(5):465–470 (1994).

39. Surks MI, Ocampo E. Subclinical thyroid disease. Am J Med 100(2):217–223 (1996).

40. Woeber KA. Subclinical thyroid dysfunction. Arch Intern Med 157(10):1065–1068 (1997).

41. Haggerty JJ Jr, Garbutt JC, Evans DL, Golden RN, Pedersen C, Simon JS, Nemeroff CB. Subclinical hypothyroidism: a review of neuropsychiatric aspects. Int J Psychiatry Med 20(2):193–208 (1990).

42. Haggerty JJ Jr, Prange AL Jr. Borderline hypothyroidism and depression. Annu Rev Med 46:37–46 (1995).

43. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 341(8):549-555 (1999).

44. Burman KD. How serious are the risks of thyroid hormone over-replacement? Thyroid Today 18(4):1–9 (1995).

45. Ross DS. Hyperthyroidism, thyroid hormone therapy, and bone. Thyroid 4(3):319–326 (1994).

46. Biondi B, Fazio S, Carella C, Amato G, Cittadini A, Lupoli G, Sacca, L, Bellastella A, Lombardi G. Cardiac effects of long-term thyrotropin-suppressive therapy with levothyroxine. J Clin Endocrinol Metab 77(2):334–338 (1993).

47. Alberti KG. The clinical implications of impaired glucose tolerance. Diabet Med 13(11):927–937 (1996).

48. Laakso M, Lehto S. Epidemiology of risk factors for cardiovascular disease in diabetes and impaired glucose tolerance. Atherosclerosis 137 Suppl:S65–S73 (1998).

49. Rezende KF, Melo A, Pousada J, Rezende ZF, Santos NL, Gomes I. Autonomic neuropathy in patients with impaired glucose tolerance. Arq Neuropsiquiatr 55(4):703–711 (1997).

50. Kaye TB. Hyperprolactinemia. Causes, consequences, and treatment options. Postgrad Med 99(5):265–268 (1996).

51. Neidhart M. Prolactin in autoimmune diseases. Proc Soc Exp Biol Med 217(4):408–419 (1998).

52. Redmond GP. Androgens in women's health. Int J Fertil Womens Med 43(2):91–97 (1998).

53. Smith KD, Rodriguez-Rigau LJ, Tcholakian RK, Steinberger E. The relation between plasma testosterone levels and the lengths of phases of the menstrual cycle. Fertil Steril 32(4):403–407 (1979).

54. Schachter M, Shoham Z. Amenorrhea during the reproductive years—is it safe? Fertil Steril 62(1):1–16 (1994).

55. McGee C. Secondary amenorrhea leading to osteoporosis: incidence and prevention. Nurse Pract 22(5):38, 41–45, 48 (1997).

56. Hergenroeder AC. Bone mineralization, hypothalamic amenorrhea, and sex steroid therapy in female adolescents and young adults. J Pediatr 126(5 Pt 1):683–689 (1995).

57. Csermely T, Halvax L, Schmidt E, Zambo K, Peterfai J, Vadon G, Szabo I. Lower bone density (osteopenia) in adolescent girls with oligomenorrhea and secondary amenorrhea. Orv Hetil 138(43):2735–2741 (1997).

58. Prior JC, Vigna YM, Barr SI, Rexworthy C, Lentle BC. Cyclic medroxyprogesterone treatment increases bone density: a controlled trial in active women with menstrual cycle disturbances. Am J Med 96(6):521–530 (1994).

59. Rodriguez MM, Grossberg GT. Estrogen as a psychotherapeutic agent. Clin Geriatr Med 14(1):177–189 (1998).

60. Kelsey JL, Whittemore AS. Epidemiology and primary prevention of cancers of the breast, endometrium, and ovary. A brief overview. Ann Epidemiol 4(2):89–95 (1994).

61. Henderson BE, Bernstein L. The international variation in breast cancer rates: an epidemiological assessment. Breast Cancer Res Treat 18 Suppl 1:S11–S17 (1991).

62. Chie WC, Hsieh C, Newcomb PA, Longnecker MP, Mittendorf R, Greenberg ER, Clapp RW, Burke KP, Titus-Ernstoff L, Trentham-Dietz A, et al. Age at any full-term pregnancy and breast cancer risk. Am J Epidemiol 151(7):715–722 (2000).

63. Hulka BS. Epidemiologic analysis of breast and gynecologic cancers. Prog Clin Biol Res 396:17–29 (1997).

64. Baker TR, Piver MS. Etiology, biology, and epidemiology of ovarian cancer. Semin Surg Oncol 10(4):242–248 (1994).

65. Meigs JB, Nathan DM, Wilson PW, Cupples LA, Singer DE. Metabolic risk factors worsen continuously across the spectrum of nondiabetic glucose tolerance. The Framingham Offspring Study. Ann Intern Med 128(7):524–533 (1998).

66. Scheen AJ. Hypercholesterolemia-related cardiovascular risk: a continuum from the notion of normality, intervention threshold and therapeutic objectives. Rev Med Liège 54(1):17–21 (1999).

67. Barrett-Connor E, Wingard DL, Criqui MH, Suarez I. Is borderline fasting hyperglycemia a risk factor for cardiovascular death? J Chronic Dis 37(9–10):773–779 (1984).

68. Naylor CD. Diagnosing gestational diabetes mellitus. Is the gold standard valid? Diabetes Care 12(8):565–572 (1989).

69. Silverman BL, Rizzo TA, Cho NH, Metzger BE. Long-term effects of the intra-uterine environment—The Northwestern University Diabetes in Pregnancy Center. Diabetes Care 21 (suppl 2):B142–B149 (1998).

70. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dorner G. Overweight and obesity in infants of mothers with long-term insulin-dependent diabetes or gestational diabetes. Int J Obes Relat Metab Disord 21(6):451–456 (1997).

71. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dorner G. Glucose tolerance and insulin secretion in children of mothers with pregestational IDDM or gestational diabetes. Diabetologia 40(9):1094–1100 (1997).

72. Roccella EJ, Boeler AE, Horan M. Epidemiologic considerations in defining hypertension. Med Clin North Am 71(5):785–801 (1987).

73. Ruilope LM, Garcia-Robles R. How far should blood pressure be reduced in diabetic hypertensive patients? J Hypertens 2(suppl 15):S63–S65 (1997).

74. Sheehan DM, Willingham E, Gaylor D, Bergeron JM, Crews D. No threshold dose for estradiol-induced sex reversal of turtle embryos: how little is too much? Environ Health Perspect 107(2):155–159 (1999).